marine drugs

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Review

Tiny Microbes with a Big Impact: The Role of Cyanobacteria and Their Metabolites in Shaping Our Future

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Abstract: Cyanobacteria are among the first microorganisms to have inhabited the Earth. Throughout the last few billion years, they have played a major role in shaping the Earth as the planet we live in, and they continue to play a significant role in our everyday lives. Besides being an essential source of atmospheric oxygen, marine cyanobacteria are prolific secondary metabolite producers, often despite the exceptionally small genomes. Secondary metabolites produced by these organisms are diverse and complex; these include compounds, such as pigments and fluorescent dyes, as well as biologically-active compounds with a particular interest for the pharmaceutical industry. Cyanobacteria are currently regarded as an important source of nutrients and biofuels and form an integral part of novel innovative energy-efficient designs. Being autotrophic organisms, cyanobacteria are well suited for large-scale biotechnological applications due to the low requirements for organic nutrients. Recent advances in molecular biology techniques have considerably enhanced the potential for industries to optimize the production of cyanobacteria secondary metabolites with desired functions. This manuscript reviews the environmental role of marine cyanobacteria with a particular focus on their secondary metabolites and discusses current and future developments in both the production of desired cyanobacterial metabolites and their potential uses in future innovative projects.

Keywords: natural products; microalgae; biotechnology

1. Introduction

Cyanobacteria are photosynthetic prokaryotes. Despite the fact they are often referred to as blue-green algae, they have no direct relation to higher algae. They are believed to be one of the oldest organisms on Earth with fossil records dating back 3.5 billion years [1,2]. Cyanobacteria are responsible for the Earth's transition from a carbon dioxide-rich atmosphere to the present relatively oxygen-rich atmosphere as a consequence of oxygenic photosynthesis [3]. Throughout their long evolutionary history, cyanobacteria have diversified into a variety of species with various morphologies and niche habitats.

Cyanobacteria present a diverse range of morph types, including unicellular, surface-attached, filamentous colony- and mat-forming species. Several species form important symbiotic associations with other micro- and macro-eukaryotes [4,5]. In keeping with the broad taxonomic diversity across the phylum, cyanobacteria inhabit a diverse range of terrestrial and aquatic habitats, ranging from deserts to freshwater and marine systems across a range of eutrophic and oligotrophic conditions. They can also be found in extreme environments, such as Antarctic dry valleys, Arctic and thermophilic

lakes [6,7], as well as in unlikely habitats for phototrophs, such as in the subsurface of calcareous rocks (*Gloeobacter violaceus*) [8] and Lava Caves [9].

Throughout their evolutionary history, cyanobacteria have developed unique interactions with other (micro- and macro-) organisms. Many of these interactions are based on a multitude of unique and complex genetic pathways leading to the production of secondary metabolites [4,5]. Secondary metabolites from cyanobacteria have been studied traditionally for their involvement in disease, e.g., microcystins and cylindrospermopsin, which trigger gastrointestinal illness, liver disease and kidney damage, or for their medicinal properties, such as anticancer, antimicrobial and UV-protective activities. The last decade has seen an increased interest in cyanobacterial research, resulting in an expansion of the uses of cyanobacterial metabolites beyond the realms of public health and pharmaceutical industries to include pigments, food and fuel production and other biotechnological applications [10,11].

Several recent publications have extensively reviewed the diversity and genetics of secondary metabolite production in (marine) cyanobacteria [12–15]. Therefore, here, we summarize this information and present insights into the current transition of research from traditional chemistry-based screens to molecular engineering and synthetic biology. These advances will not only contribute to basic knowledge, but will also further drive the use of cyanobacterial secondary metabolites in novel applications.

2. Environmental Impact of Marine Cyanobacterial Secondary Metabolites

Some of the earliest research on cyanobacterial secondary metabolites derived from the study of toxins produced by harmful algal blooms (HAB) and was mainly focused on freshwater species [16–18]. Toxin production by HAB can have dramatic health and economic impacts in lakes, rivers, estuarine and coastal shores, resulting in the death of cattle and domestic animals, as well as shellfish poisoning, leading to substantial financial loss to industries (Figure 1) [19].

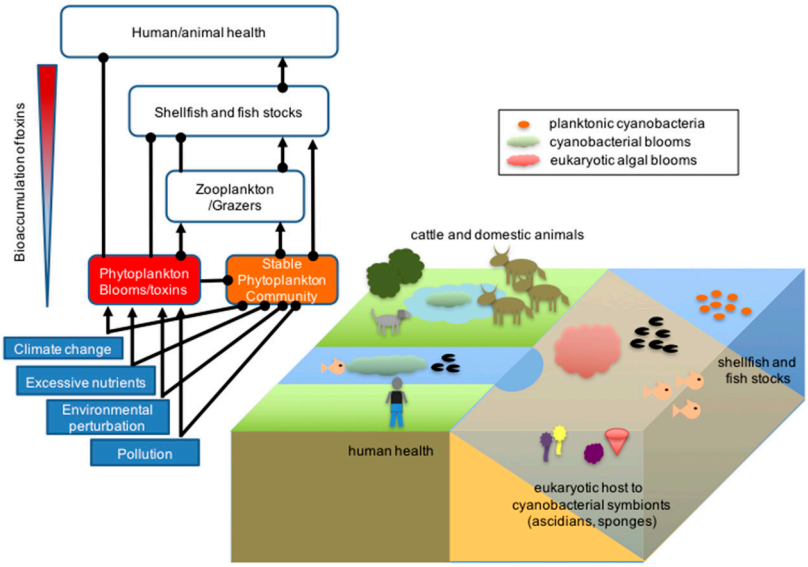

Figure 1. Environmental impact of photosynthetic microorganisms in aquatic systems. Different classes of photosynthetic microorganisms are found in aquatic and marine environments where they form the base of healthy food webs and participate in symbioses with other organisms. However, shifting environmental conditions can result in community dysbiosis, where the growth of opportunistic species can lead to harmful blooms and toxin production with negative consequences to human health, livestock and fish stocks. Positive interactions are indicated by arrows; negative interactions are indicated by closed circles on the ecological model.

The structure, cellular target and bioactivity of HAB toxins are broad and include soluble compounds of several types, such as neurotoxins, hepatoxins, cytotoxins, dermatoxins, in addition to endotoxins, e.g., lipopolysaccharides (LPS). The best-studied examples of cyanobacterial toxins are the neurotoxins; anatoxin-a/saxitoxin (*Anabaena flos aquae*) [20,21] and the potent hepatotoxin microcystins (*Microcystis* sp.) [22]. However, while some of these toxin-producing freshwater cyanobacterial species can expand into estuarine environments, it is interesting to note that toxin-producing unicellular species rarely predominate in truly marine habitats [18]. In the marine environment, toxin production appears limited to the filamentous colony-forming cyanobacteria, Oscillatoriales, *Trichodesmium, Lyngbya* (reclassified as *Moorea* sp. [23]) and *Nodularia*, and the (phyto) planktonic dinoflagellates and diatoms. Indeed, similar to freshwater cyanobacteria, these species form recurrent seasonal outbreaks leading to toxic blooms affecting shellfish and finfish stocks with dramatic consequences for aquaculture and human consumers [24]. The greater occurrence of HABs in estuarine and coastal waters has been linked to increased eutrophication, in particular nitrogen and phosphorus loading due to runoff from agricultural land. In recent times, greater public awareness and better agricultural management practices in many developed countries have reduced the occurrence of nutrient-induced HABs. However, ecosystem perturbations, such as localized heat waves, and habitat stress from human activities, including aquaculture, urbanisation and shipping, are increasingly linked to recurrent HABs [17,25], potentially as a result of the dysbiosis of microbial communities that form the base of healthy marine ecosystems.

Several marine cyanobacteria produce toxins, although these genera appear less prevalent in oceanic compared to coastal settings. Marine cyanobacterial blooms are more prominent in tropical and sub-tropical regions, mainly in shallow reef areas. The main bloom-forming species include *Synechocystis*, *Oscillatoria*, *Lyngbya* (*Moorea* [23]) and *Symploca*. Relative to their freshwater counterparts, toxins produced by marine cyanobacteria are thought not to present a direct health risk, mainly due to the fact that humans and domestic animals do not rely on seawater for drinking. However, they can lead to secondary health risks through bioaccumulation or poisoning of fishes and other seafood. To date, the major human health risk of marine cyanobacteria has been associated with members of the genera Oscillatoriales, *Moorea* and *Trichodesmium*. For example, *Lyngbya majuscula (Moorea producens)* is a prolific producer of diverse secondary metabolite compounds, including lyngbyatoxins and majusculamides. These marine cyanobacterial toxins have a broad range of biological activities, including dermatotoxic, cytotoxic, neurotoxic and tumorigenic activities [12,18].

Specific environmental conditions, especially enriched nutrient conditions, such as phosphorus and iron, promote the growth and formation of mats and coastal blooms attributed to *Lyngbya/Moorea* [26]. During these times, the overgrowth of the cyanobacteria and toxin production have become the cause for the closure of beaches partly due to the presence of skin irritant dermatoxin known to cause "swimmers' itch" [27]. These outbreak events lead to reduced public confidence in seafood and equally damage the tourism industry. Estimates drawn in the U.S. state that harmful algal blooms (HABs) were costing approximately US$100 million per year to the U.S. economy in lost fishery production and stocks, human illness and lost tourism revenue [28], totalling upwards of US$1 billion during the past decades [29,30]. In Australia, the negative impact of cyanobacterial HABs was estimated to cost $180–240 million per year [31,32], with some blooms of photosynthetic microbes hypothesized to dramatically affect local businesses. Indeed, blooms of *Nodularia* and specifically *N. spumigena*, a brackish heterocystous genus producing hepatotoxin nodularin, have repeatedly caused issues around Australia and appear to be gradually expanding their biogeography [33,34]. In September 2008, one such bloom at a brackish lake in Queensland forced the closure for recreational access of a cable ski operation for a duration of three months at an estimated cost of AUD$300,000 [34].

Interestingly, the genome sequencing of *L. majuscula* (*Moorea producens*) suggested that it uses precursors from other surrounding bacteria to synthesise a proportion of its toxins [35–37]. Therefore, *Moorea's* toxicity could be the result of a network association with metabolic exchanges between the various individuals in the microbial community. Thus, in order to mitigate the negative impacts

of marine cyanobacterial toxins, it is important study these organisms within the appropriate ecological context.

3. Ecological Role of Marine Cyanobacterial Secondary Metabolites

Marine cyanobacteria can be found in various environmental niches, both as pelagic free-living forms and in the benthos, either forming mats on surfaces, or as symbionts of eukaryotes, such as sponges, ascidians or kelps. The benthic or host-associated forms of marine cyanobacteria appear to be a richer source of complex bioactive secondary metabolites, likely due to the character of this ecological niche, which facilitates a highly competitive and relatively nutrient-rich environment provided by the host [38,39]. Notably, multiple compounds, which were originally thought to be produced by higher organisms, such as sponges and ascidians, such as dolastatin and analogues (sea hare) leucamide A (sponge) and westiellamide (tunicate), are now shown to be synthesized by an associated cyanobacterium [12,37,40,41]. These marine cyanobacteria live in a complex ecosystem defined by close associations and intense competition from other members of the community and a higher frequency of encounters with numerous predators, including grazers and phage. Many of the metabolites they produce are thought to play an important part of defence mechanisms to attempt to gain the upper hand and thrive within their niche of choice.

Some marine cyanobacteria produce small molecules with structural similarity to compounds involved in bacterial quorum sensing, such as acyl-homoserine lactones [14]. These molecules act as inhibitors of bacterial quorum sensing; however, their mode of action is unclear, as, despite their structural similarity to known acyl-homoserine lactones, the cyanobacterial compounds were not shown to act as direct competitive inhibitors [42]. For example, *Lyngbya* (consisting probably of the renamed *Moorea* sp.) is known to proliferate in dense microbial mats and to produce several interfering metabolites, such as malyngamide, malyngolide and lyngbyoic acid [43–46]. In this habitat, the production of quorum sensing-interfering compounds may provide an advantage by interfering with regulatory networks of competitors [36]. Many of these compounds from marine organisms, including not only cyanobacteria, but also algae, fungi, tunicates and sponges (many secondary metabolites of which could be the result of cyanobacterial symbionts), have attracted commercial interest as they could prove useful in preventing marine biofouling through bacterial quorum sensing inhibition, as was shown for Microlins A and B from *L. majuscula* (now *Moorea* sp.) [47]. Planktonic marine cyanobacteria have also been reported to produce allelopathic compounds to gain advantage in some habitats. For example, *Synechococcus* CC9605, a coastal-dwelling cyanobacterium, has been shown to produce microcin C-like metabolites that inhibit the growth of other cyanobacteria strains [48], and marine *Cyanobium* strains produce bioactive compounds against a range of other marine organisms [49].

Due to their abundance and role as the base of the many aquatic food chains, cyanobacteria are constantly consumed by larger planktonic microbes, filter feeders and grazers. Hence, it is not surprising that cyanobacteria have developed effective chemical deterrents [36]. These molecules, which act as herbivore deterrents, are produced by benthic marine cyanobacteria and are excreted or exported to alter cell surface properties that lower their palatability to predators. Many have no demonstrated toxicity, but may act as repellents, leading to starvation of the grazer by removing their only food source, as these grazers will not feed on the cyanobacterial mat. For example, production of ypaoamide (Figure 2) by the assemblage of *Schizothrix calcicola* and *L. majuscula* acts a as deterrent to macrograzers, such as rabbitfish and sea urchins [50], and other yet unknown chemical deterrents from *L. majuscula* act against various grazers, such as sea urchins, crabs and other amphipods [51]. Despite these efficient deterrent mechanism, some mesograzers still feed on toxic cyanobacteria and have succeeded to adapt cyanobacterial defence systems for their own use. Indeed, sea hares accumulate large amounts of metabolites within their tissues, which are hypothesized to be derived from their cyanobacterial diet [52].

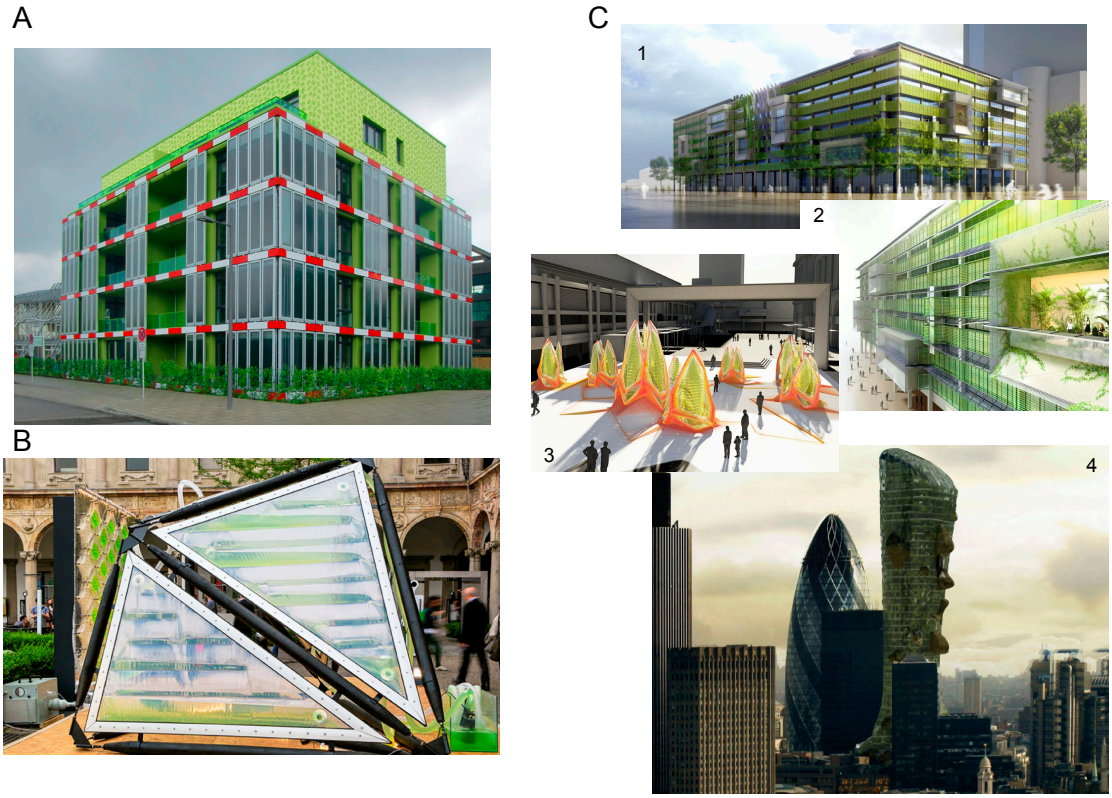

Figure 3. (**A**) Bio Intelligent Quotient (BIQ)—The Clever Treefrog—The Algaehouse, housing-project at the IBA Hamburg. Idea, concept and authorship: SPLITTERWERK, Label for Fine Arts, Graz; Arup GmbH, Berlin; B+G Ingenieure Bollinger und Grohmann GmbH, Frankfurt; Immosolar GmbH, Hamburg. Photo by SPLITTERWERK 2013. (**B**) Urban Algae Façade prototype by Cesare Griffa and Carlo Ratti Associati; prototyping team: Matteo Amela, Federico Borello, Marco Caprani; technical support by Environment Park Spa, Fotosintetica & Microbiologica Srl; lighting by iGuzzini. Photos by Filippo Ferraris. 2014 Salone del Mobile, Milan, Italy. (**C1**) Process Zero Exterior: the eight-story, 1960s-era building is among the 362 million square feet of office space the GSA must retrofit to reduce greenhouse gases by 30 percent before the 2020 deadline. Image credit: HOK/Vanderweil. (**C2**) Process Zero Exterior Facade Detail: algae, housed in glass tubes covering the building's exterior, filters wastewater, consumes carbon dioxide from the nearby highway and uses photosynthesis to produce energy. Image credit: HOK/Vanderweil. (**C3**) Concept image created for the Perth Photobioreactor Design and copyright to Tom Wiscombe architecture. (**C4**) The FMSA Tower concept project, a self-sustainable skyscraper. Design and copyright to Dave Edwards. All photographs and artwork reproduced with permission of the authors.

5. Conclusions

Since much of the attention in relation to metabolite production has been historically focused on their freshwater counterparts, marine cyanobacteria present a relatively untapped resource in terms of evolutionary diversity and industrial potential. They are prolific producers of diverse and complex secondary metabolites with potential applications in health, biofuels and bioengineering. They have minimal genomes and low cellular resource requirements, which make them well suited for genetic and metabolic engineering. In light of demands on natural resources, including freshwater, nutrients and arable land, marine cyanobacteria offer an important advantage over their freshwater counterparts for industrial-scale processes, *i.e.*, they are adapted to growing in brackish and salt water. Coupled with their ability to convert sunlight to energy, these organisms have the capacity to serve as low cost, adaptable cellular factories capable of producing high-value products and biofuels with low environmental impact.

Cyanobacteria and, particularly, the marine dwellers have become increasingly integral parts of future innovative projects, from aeronautic programmes to concept projects in sustainable architecture. The incorporation of algae into novel architectural designs has the potential to improve waste recycling, climate control and reduce the carbon footprint of commercial buildings. Much is still unknown about marine cyanobacterial metabolites; however, there is a great deal of progress being made using recent advances in molecular techniques, including large-scale environmental genome sequencing projects, metabolic modelling and synthetic biology approaches. Expanding the potential biotechnological benefits of marine cyanobacteria will benefit from collaborations across the fields of ecology, genomics, chemistry, health research and engineering and will result in the development of new technologies, including extending the range of cyanobacterial metabolites beyond traditional uses, optimizing biofuel production by using non-arable land and abundant saline water resources and contributing to the ecological buildings of the future.

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Abbreviations

The following abbreviations are used in this manuscript:

CO_2	carbon dioxide
FMSA	financial market service authority
GSA	general services administration
HIV	human immunodeficiency virus
HOK	formerly Hellmuth, Obata + Kassabaum, design firm
IBA	International Building Exhibition
N	nitrogen
P	phosphorus
R&D	research and development
U.S.	United States (of America)
UV	ultra-violet

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